

Ultrastructural description of *Dientamoeba fragilis* and a new viral-like particle

By

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A thesis submitted in fulfillment of the requirements for the
degree of Doctor of Philosophy



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2014

Certificate of original authorship

This study was conducted in the School of Medical and Molecular Biosciences and i3 institute, Faculty of Science, University of Technology, Sydney and in the Microbiology Department, St. Vincent's Hospital Sydney, under the supervision of Professor John T. Ellis and Dr. Damien Stark.

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me with editorial help from Prof. John Ellis and Dr. Damien Stark as acknowledged in individual chapters. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. Finally, I certify that all information sources and literatures used are indicated in the thesis.

Gouri Rani Banik

March 2014

Acknowledgements

This thesis is an outcome of my everyday work under the supervision of Prof. John Ellis and Dr. Damien Stark. At the very beginning, I would like to gratefully acknowledge both my supervisors for all their direction, patience, constructive feedback, expert guidance and encouragement throughout my candidature which were invaluable to my study. It would not be achievable without their continuous support. Their valuable suggestions, contributions and supports are greatly appreciated.

Special thanks to Ms Debra Birch for her special guidance during my work at Macquarie. I wish to thank her for valuable comment on relevant paper. I also would like to thank Ms Nicole Vella and Dr Michael Johnson for their technical suggestions during my work.

I wish to acknowledge all the team members in Professor Ellis's lab for their assistance in every way especially Joel Barratt, Varuni Munasinghe, Stephanie Fletcher and Tamalee Roberts. I also thank Andrew Liew for his unconditional support all the time. Also my other lab colleagues Heba, Jen and Atik for supporting me and make my PhD life really enjoyable.

An individual thanks goes to Professor Steven Djordjevic and Dr. Matthew Padula for their valuable suggestions during my protein work and Mr. Philip Lawrence, Mr. Harry Simpson, Mr. Rowan Ikin and Dr. Ian Garthwaite for their everyday help in the lab. Many thanks to Dr Lisa Sedger for her valuable suggestions.

I am grateful to ithree institute and University of Technology, Sydney for giving me the opportunity to conduct my study and I do appreciate their financial support during my study.

Last but not least my gratitude goes to my beloved husband Palash for his everyday support and understanding whenever I was distressed. Thank you for your never- ending faith in me. My appreciation goes to mom, dad and my brother for their unconditional love, encouragement and endless support. My beloved daughter Anvi and Aarna deserves a very special mention, who has sacrificed a lot so that I could achieve my goal.

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3. **Banik, G. R.**, Birch , D., Stark, D., Ellis, J. T., 2012. A microscopic description and ultrastructural characterisation of *Dientamoeba fragilis*: An emerging cause of human enteric disease. *International Journal for Parasitology*, 42: 139-153.
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4. **Banik, G. R.**, Birch, D., Stark, D., Ellis, J. T., 2013. Virus-like particles (VLPs) in *Dientamoeba fragilis*: an ultrastructural study (Submitted for publication in the *Journal of Parasitology*, November 2013).

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- **Banik, G. R.**, Barratt. J. L.N., Marriott, D., Harkness, J., Ellis, J. T., and Stark, D. A case-controlled study of *Dientamoeba fragilis* infections in children, **Poster presentation**, ICOPA, Melbourne, Australia, 15 -19th August, 2010.

- **Banik, G. R.**, Barratt. J. L.N., Marriott, D., Harkness, J., Ellis, J. T., and Stark, D. A case-controlled study of *Dientamoeba fragilis* infections in children, **Poster presentation**, 27th RNSH.UTS.USYD. Kolling Scientific Reserach Meeting, Sydney, Australia, 9-10th November, 2010.

- **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. A microscopic description and ultrastructural characterisation of *Dientamoeba fragilis*: An emerging cause of human enteric diseases. **Poster presentation**, 28th RNSH.UTS.USYD. Kolling Scientific Reserach Meeting, Sydney, Australia, 1-2nd November, 2011.

- **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. Electron microscopy characterisation of *Dientamoeba fragilis* virus life cycle. **Oral presentation**, Australian Society for Parasitology Annual Conference, Launceston, Tasmania, 2-5th July, 2012.

- **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. Electron microscopy characterisation of *Dientamoeba fragilis* virus-like particles, **Poster presentation**, 29th RNSH.UTS.USYD. Kolling Scientific Reserach Meeting, Sydney, Australia, 20 -21st November, 2012.

- **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. Electron microscopy characterisation of *Dientamoeba fragilis* virus-like particles, **Oral and Poster presentation**, Gordon Research Seminar and Conference on Physical Virology, Ventura, California, USA, 19-25th January, 2013.

■ **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. Electron microscopy characterisation of *Dientamoeba fragilis* virus-like particles, **Poster presentation**, New Horizons 2013, 30th Combined Health Science Conference, Kolling Building, Royal North Shore Hospital, NSW, 18 -20th November, 2013.

Abbreviations

Terms:

| | |
|-------|-------------------------------------|
| Ax | Axostyle |
| ATCC | American Type Culture Collection |
| BB | Basal Body |
| cDNA | Complementary Deoxyribonucleic Acid |
| Ch | Chromatin Bodies |
| Co | Costa |
| CP | Capsid Protein |
| CsCl | Caesium Chloride |
| DAPI | 4', 6-diamidino-2-phenylindole |
| DFV | <i>Dientamoeba fragilis</i> Virus |
| DNA | Deoxyribonucleic Acid |
| DNase | Deoxyribonuclease |
| DIC | Differential Interference Contrast |
| dsRNA | Double Stranded Ribonucleic Acid |
| Dv | Digestive Vacuole |
| ED | Electron Dense |
| EDTA | Ethylenediaminetetraacetic Acid |
| EGTA | Ethylene Glycol Tetraacetic Acid |
| EM | Electron Microscopy |
| ENV | <i>Eimeria necatrix</i> Virus |
| ER | Endoplasmic Reticulum |
| ESV | <i>Eimeria stiedae</i> Virus |
| EtOH | Ethyl Alcohol |

| | |
|-------------------|-------------------------------|
| Gc | Golgi Complex |
| GFP | Green Fluorescent Protein |
| GLV | <i>Giardia lamblia</i> Virus |
| HCl | Hydrochloric Acid |
| HIV | Human Immunodeficiency Virus |
| ITS | Internal Transcribed Spacer |
| LRV | <i>Leishmania</i> RNA virus |
| MgCl ₂ | Magnesium Chloride |
| My | Myelin Sheath |
| Mt | Microtubules |
| MTOC | Microtubule Organizing Center |
| Nm | Nuclear Membrane |
| NaCl | Sodium Chloride |
| Np | Nuclear Pore |
| ORF | Open Reading Frame |
| OsO ₄ | Osmium Tetroxide |
| PBS | Phosphate Buffered Saline |
| PCR | Polymerase Chain Reaction |
| PEG | Polyethylene Glycol |
| Pf | Parabasal Filament |
| Pm | Plasmalemma |
| Ps | Pseudopodia |
| PVA | Polyvinyl Alcohol |
| RNA | Ribonucleic Acid |
| RNase | Ribonuclease |

| | |
|----------|---|
| RdRp | RNA Dependent RNA Polymerase |
| Rs | Rice Starch |
| RT-PCR | Real-time Polymerase Chain Reaction |
| ssRNA | Single Stranded Ribonucleic Acid |
| SAF | Sodium Acetate -Acetic Acid- Formalin |
| ScV | <i>Saccharomyces cerevisiae</i> Virus (ScV) |
| S.D. | Standard Deviation |
| SDS | Sodium Dodecyl Sulfate |
| SEM | Scanning Electron Microscopy |
| ssRNA | Single-Stranded Ribonucleic Acid |
| SSU rRNA | Small Subunit Ribosomal RNA |
| sv | Small Vacuole |
| TBE | Tris Borate EDTA |
| TE | Tris-EDTA |
| TEM | Transmission Electron Microscopy |
| TM | Tris- $MgCl_2$ |
| tRNA | Transfer RNA |
| TVV | <i>Trichomonas vaginalis</i> Virus |
| UTR | Untranslated Region |
| VLP | Virus-Like Particle |
| WSSV | White Spot Shrimp Viruses |

Units:

| | |
|----|----------------------------|
| °C | Degree Celsius |
| g | Relative Centrifugal Force |

| | |
|-----|--------------|
| h | Hour |
| Kb | Kilobase |
| KDa | Kilo Daltons |
| Kg | kilogram |
| M | Molar |
| Mb | Megabase |
| μM | Micromolar |
| μm | Micrometre |
| μg | Microgram |
| μL | Microlitre |
| mg | Milligram |
| mL | Millilitre |
| mM | Millimolar |
| min | Minute |
| ng | Nanogram |
| nm | Nanometer |
| U | Unit |

Abstract

Dientamoeba fragilis is a trichomonad protozoan found in the gastrointestinal tract of humans and is implicated as a cause of diarrhoeal disease. Despite its widespread occurrence and associated symptoms, very little is known about the biology and pathogenicity of *D. fragilis*. Advances in cell biology of other trichomonads means there is a need to advance knowledge on this neglected protozoan.

In this study, the morphological characteristics and ultrastructure of *D. fragilis* were described in detail using different microscopy techniques. Scanning electron microscopy, transmission electron microscopy, confocal and light microscopy were used to characterise *D. fragilis* populations growing in xenic culture. Under the SEM, two types of *D. fragilis* populations were identified based on cell surface structure: smooth cells and ruffled cells. Typically *D. fragilis* has a spherical or oval shape with a granular, vacuolated cytoplasm and some cells are amoeboid. *Dientamoeba fragilis* exhibited different motile forms with visible pseudopodia. The organelles in *D. fragilis* were analysed by transmission electron microscopy; the pelta, flagella, undulating membrane or pseudocyst-like forms were not found. The presence of hydrogenosomes in *D. fragilis* is described which has not been previously reported. The majority of cells grown in culture were mononucleate while most cells in permanent stained faecal smears were binucleate. Evidence is presented using confocal microscopy that the two nuclei of *D. fragilis* are identical in DNA content. In addition, the discovery of a virus-like particle is reported for the first time in *D. fragilis*. This study provides extensive and new detail on the ultrastructure of *D. fragilis* that is an emerging cause of human enteric disease.

Dientamoeba fragilis virus (virus-like particles or VLPs) was studied further: it was approximately 33-40 nm in size and the most common shape was spherical. These VLPs contain an inner dark core surrounded by an electron-lucent layer and an electron dense capsid coat. Virus particles are found extensively in the perinuclear region of the trophozoite, and especially around microtubules and in association with the Golgi complex. Virus particles were also found in the vicinity of endoplasmic reticulum, axostyle, and near to the parabasal filament but no VLPs were found in the nucleus.

Dientamoeba fragilis VLPs were also detectable in dying trophozoites present in *in vitro* cultures. Whether viral load contributes to cell death is not yet known.

The identity of the *D. fragilis* viral genome was also studied. Several different extraction methods were screened and three different methods were optimized to identify dsRNA from *Trichomonas vaginalis* (B7268 isolate) which was used as a positive control for the isolation of viral dsRNA. These optimized methods were evaluated to identify *D. fragilis* viral genome. No viral RNA or dsRNA was isolated from *D. fragilis* suggesting that unlike *T. vaginalis*, *D. fragilis* trophozoites do not contain a dsRNA virus, or that the abundance of the virus was so low that it prevented the identification of viral nucleic acid.

The epidemiology of *D. fragilis* has not been studied in detail and as a small side project I investigated hospital records for infections of children. Consequently, a case-controlled study was conducted to document the extent of *D. fragilis* infections in children presenting to a major Sydney Hospital. Treatment options are also discussed. In total, hospital data from 41 children were included in the study along with a control group. Results showed that diarrhoea (71%) was found to be the most common symptom followed by abdominal pain (29%). In addition, diarrhoea was statistically more significant in children with *D. fragilis* infection compared to a control group. In this study, the most common antimicrobial used for treatment was metronidazole (n=41), with complete resolution of symptoms and clearance of parasite occurring in 85% of cases. Moreover, a treatment failure rate of 15% was identified in children treated with metronidazole. These studies further suggest the pathogenic nature of *D. fragilis* and it is recommended that all laboratories must routinely test for *D. fragilis* as treatment which eliminates the parasite usually results in the resolution of symptoms.

In summary, this thesis has discussed many novel aspects on the biology of *D. fragilis* and provide new knowledge on the cell biology of this protozoan and a new protozoan virus.